Prospective study into the value of the automated Elecsys antimüllerian hormone assay for the assessment of the ovarian growing follicle pool

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Objective: To evaluate a new fully automated assay measuring antimüllerian hormone (AMH; Roche Elecsys) against antral follicle count in women of reproductive age.
Design: Prospective cohort study.
Setting: Hospital infertility clinics and academic centers.
Patient(s): Four hundred fifty-one women aged 18 to 44 years, with regular menstrual cycles.
Intervention(s): None.
Main Outcome Measure(s): AMH and antral follicle count (AFC) determined at a single visit on day 2–4 of the menstrual cycle.
Result(s): There was a statistically significant variance in AFC but not in AMH between centers. Both AFC and AMH varied by age (overall Spearman rho −0.50 for AFC and −0.47 for AMH), but there was also significant between-center variation in the relationship between AFC and age but not for AMH. There was a strong positive correlation between AMH and AFC (overall spearman rho 0.68), which varied from 0.49 to 0.87 between centers. An agreement table using AFC cutoffs of 7 and 15 showed classification agreement in 63.2%, 56.9% and 74.5% of women for low, medium, and high groups, respectively.
Conclusion(s): The novel fully automated Elecsys AMH assay shows good correlations with age and AFC in women of reproductive age, providing a reproducible measure of the growing follicle pool. (Fertil Steril® 2015;103: 1074–80. ©2015 by American Society for Reproductive Medicine.)
Key Words: Antimüllerian hormone, antral follicle count, ovarian follicle, ovarian reserve, reproductive life span

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Prediction of response and outcome in assisted reproduction is a central aspect of current practice, allowing greater individualization of treatment protocols, reducing the risk of potentially serious adverse effects such as ovarian hyperstimulation syndrome (OHSS), and, at the other end of the spectrum of response, identifying poor responders and thus overall providing more accurate information to patients. The main approach to such prediction is analysis of ovarian

biomarkers that reflect follicular activity and thus the response to gonadotropins during subsequent ovarian stimulation. To achieve maturity during the time course of stimulation, ovarian follicles must already be at the small antral stage of development, so clinically valuable biomarkers will accurately quantify the number of such growing follicles, often termed the ovarian reserve. Although several biomarkers have been investigated over the years (including follicle-stimulating hormone [FSH], estradiol, and inhibin B), two have shown markedly greater accuracy and are in widespread use: antral follicle count (AFC) and antimüllerian hormone (AMH) (1–4). These have been extensively investigated in women undergoing assisted conception but have much broader application in women's health across the reproductive life span.

Transvaginal ultrasound is used to determine AFC, identifying and counting the number of small antral follicles. Developments in technology have improved image resolution, allowing follicles $\geq$2 mm to be readily visualized; follicles up to 10 mm are generally included in the analysis. The normal range is currently a matter of controversy (5). Although prediction of pregnancy is poor, AFC shows good prediction of the number of oocytes that will be retrieved after stimulation (3, 6). Its immediacy and wide availability are significant advantages. However, although automated determination of AFC is being developed and protocols for standardization are described (7), it remains an essentially subjective measure affected by the operator, the equipment used, and the patient because factors such as high body mass index (BMI) and pelvic pathology may impact the result.

Measurement of circulating AMH also reflects the number of small antral follicles and is predictive of ovarian response (1, 6). Although AMH is produced by the granulosa cells of follicles from the earliest stages of growth through the preantral and early antral stages, production declines abruptly at the stage at which follicles are selected for dominance, 8–10 mm (8). In normal women, the population of follicles of 5–8 mm diameter produces most circulating AMH (9). In comparison with AFC, AMH shows good prediction of oocyte number and similarly limited prediction of pregnancy and live birth (4, 6, 10). As a biochemical test, there are potential advantages in standardization and thus consistency in results both within and between centers, but this has yet to be realized. The currently available assays are manual, plate-based enzyme-linked immunosorbent assays (ELISAs); although these have led to a wealth of understanding of the value of AMH measurement across a wide range of physiologic and clinical situations (11), there are issues of lack of standardization among those produced by different manufacturers and concerns about data reliability (12).

Fully automated platform-based AMH assays are being developed, with the characteristics of one developed by Roche Diagnostics recently described (13). Our study was designed to investigate the value of AMH measurement using this novel Elecsys AMH assay in the assessment of ovarian reserve as expressed by AFC.

MATERIALS AND METHODS

Study Design

Our prospective observational study, undertaken in seven infertility centers, enrolled a subject cohort of healthy female volunteers and patients and measured the AMH concentration in relation to the AFC determined via transvaginal sonography on days 2–4 of a menstrual cycle. The women were recruited from the general population and from infertility clinics. In both cases, the inclusion criteria included ages 18–44 years inclusive, regular self-reported menstrual cycles from 24 to 35 days in length, and informed consent given in writing. The exclusion criteria included pregnancy, major uterine or ovarian abnormalities detected by transvaginal sonography, polycystic ovary syndrome (PCOS), endocrine or metabolic abnormalities (i.e., diabetes type I or II, or pituitary, adrenal, pancreas, liver, or kidney disturbances), ovarian surgery in the past 6 months, hormone therapy in the preceding 3 months (hormonal contraceptives, gonadotropin-releasing hormone [GnRH] agonists, FSH), or current or past smoking. The sample size calculation was based on accurate estimation of the Spearman correlation coefficient as well as on an accurate estimation of AMH cutoffs for the AFC $<$7 and AFC $\geq$15 group.

We calculated that at least 400 patients should be enrolled to obtain accurate estimates for the Spearman correlation coefficient as well as AMH cutoffs. With this number of patients, a preassumed correlation coefficient of 0.6 can be estimated with the width of its confidence interval smaller than 0.2 (14). In addition, the assumed prevalences for the AFC $\leq$7, AFC 8–15, AFC $>$15 groups were 10%, 40%, and 50%, resulting in the determination of the 10% and 50% quantile for AMH cutoffs. With the determined sample size, the width of the confidence intervals (CI) of these cutoffs will be smaller than 0.56 ng/mL. The study received ethics committee approval in all centers.

The study intervention consisted of a single visit, at which a blood sample was taken for later hormone measurements and a transvaginal ultrasound examination was performed to determine AFC. Antral follicles were classified as those measuring 2–10 mm in diameter, and AFC was determined as the overall number of antral follicles counted in both ovaries. In all centers, AFC was determined by as few individuals as possible, and a consistent methodology was followed (7). In all centers, two-dimensional (2D) transvaginal ultrasound equipment with a probe $\geq$6 MHz, minimal resolution 2 mm was used (Supplemental Table 1, available online). The sonographic evaluation of all study participants was performed between January 2013 and January 2014.

Blood was allowed to clot, and the serum separated by centrifugation and stored at −80°C (or at −20°C for a maximum of 6 months) until analysis after shipping to a central laboratory (Free University Brussels, Belgium). All serum markers (AMH, FSH, E_2) were determined in single measurement on the e601-module of the fully automated cobas® 6000 system. The measurements were split over 10 independent runs which were performed on different days. For each of the markers, a two-level control sample set was determined

in each run (AMH 0.93 and 4.8 ng/mL, FSH 17.5 and 44.5 IU/L, and E_2 368 and 1,999 pmol/L).

The new Elecsys AMH assay is a sandwich assay based on electrochemiluminescence technology. The total duration of the assay is 18 minutes, and the sample volume is 50 μL. The assay is calibrated against the Beckman Coulter AMH Gen II ELISA (unmodified version without predilution) assay with a measuring range of 0.01–23 ng/mL. The limit of quantitation (functional sensitivity) is 0.03 ng/mL. The coefficients of variation as determined for the control samples during the study measurements were $\leq$3.3%, $\leq$2.2%, and $\leq$3.7% for the intermediate precision for AMH, FSH, and E_2, respectively. The statistical analysis was performed using R 3.0.1 software.

The influence of categorical covariables on the AMH or AFC value was based on analysis of variance (ANOVA) F tests, and box plots show the distribution of these values in the different categories. Individual group comparisons were based on the *t* test. The correlation between AMH or AFC and other continuous variables as well as between AMH and AFC was assessed via Spearman's rho correlation coefficient and tested whether this correlation coefficient is different from zero. The relationship between AFC and age, as well AMH and age, is exemplified by the Passing-Bablok regression, a robust regression analysis. The agreement between AFC and AMH groups (low AFC group $\leq$7; middle AFC group = 8–15; high AFC group >15) (15, 16) is shown in an agreement table, with absolute numbers as well as percentages. Receiver-operator characteristic (ROC) curves are used to show the classification potential of AMH to identify low ovarian reserve and high ovarian reserve based on AFC <7 and AFC >15, respectively. Sensitivity, specificity, and Youden's index were calculated for the AMH quantiles as derived for the agreement tables. For ROC curves, the area under the curve (AUC) with its 95% confidence interval (CI) was calculated as well. For the combination of AMH and age, a logistic regression model was used for the classification in low or high ovarian reserve.

RESULTS

For our study, 487 eligible women met the inclusion and exclusion criteria. From these women, 36 had to be excluded because of sample-handling issues, so data from 451 women were used for the statistical analysis, with the number of women contributed by each site varying between 115 and 17. Their demographic characteristics are given in Table 1. As for the participating women, 92% were Caucasian, their mean age was 32.8 years (range: 18.0–44.0 years), their mean BMI was 24.3 kg/m^2 (range: 16.7–52.7), and 68.5% were not infertile. Their ages were approximately evenly distributed between groups aged 18–29, 30–34, and 35–39 years, with fewer aged 40–45 years.

To minimize interobserver variation, 91% of AFC determinations were performed by 13 clinicians/sonographers. The distribution of AFC and AMH values is shown in Supplemental Table 2 (available online) overall and for each site, together with FSH and E_2 measurements. There were statistically significant differences in mean AFC values among the centers ($P<.001$) whereas the AMH mean values did not vary by center ($P=.30$). As expected, both AFC and AMH varied by age (overall Spearman's rho −0.50 for AFC and −0.47 for AMH, $P<.001$; Fig. 1). There was also statistically significant between-center variation for age-adjusted AFC ($P<.001$) but not for age-adjusted AMH ($P=.19$).

The primary aim of this study was to investigate AMH (using the Elecsys AMH assay) as a biomarker of the ovarian reserve determined by AFC. The study confirmed that there is a strong positive correlation between AMH and AFC (Spearman's rho = 0.68, $P<.001$). This relationship was present in each center (all $P<.001$; Supplemental Table 3, available online), although Spearman's rho varied from 0.49 to 0.87.

As AFC is used clinically to define poor responders and those at risk of OHSS, further analysis explored AMH by AFC group using the AFC groupings of 0–7, 8–15, and >15 (15). This showed highly significant differences in mean AMH between the AFC groups ($P<.001$; Supplemental Fig. 1, available online). An agreement table was constructed using the same AFC cutoffs of 7 and 15, with an AFC of 7 corresponding to the 15th percentile and AFC of 15 to the 52nd percentile. The equivalent percentiles for AMH were 0.68 ng/mL and 2.27 ng/mL. This analysis (Table 2) shows classification agreement in 63.2%, 56.9%, and 74.5% of women for the low, medium, and high groups, respectively.

We also analyzed AMH in comparison with FSH and E_2 concentrations, with all samples being taken on days 2–4 of the menstrual cycle. The FSH concentrations ranged from 0.51 to 45.9 IU/L and E_2 from 18.4 to 684 pmol/L. There was a negative relationship between AMH and FSH (Spearman's rho −0.42, $P<.001$) but no statistically significant relationship with E_2 (Spearman's rho −0.04, $P=.45$).

The ROC curve analysis was performed for the classification of low AFC ($\leq$7), which included 66 women versus 385 with AFC >7 and high AFC >15 (216 women vs. 235 women with lower AFC) (Fig. 2). For both low and high AFC classifications, AMH showed good discrimination with AUC of 91.1% (95% CI, 87.1%–95.2%) and 82.7% (95% CI, 79.0%–86.5%), respectively (both $P<.001$). This was statistically significantly better than for age, and markedly so than for the hormones FSH and E_2. The sensitivity and specificity calculated for the classification of low and high AFC by means of the AMH quantiles as derived for the agreement tables were 65.2% and 93.5%, respectively, for the low AMH quantile (0.68 ng/mL), and 74.5% and 76.2% for the high AMH quantile (2.3 ng/mL). Youden's indices (sensitivity + specificity −1) were 0.59 and 0.51, respectively. The combination of AMH with age did not statistically significantly improve the clinical performance for the low and high AFC classifications: AUC 91.7% (95% CI, 87.8%–95.6%) and 83.6% (95% CI, 80.0%–87.2%), respectively.

DISCUSSION

This study shows the value of a novel fully automated Elecsys AMH assay in the analysis of ovarian reserve as defined by AFC. Both AFC and AMH have become widely used biomarkers for what is widely termed the ovarian reserve in the context of prediction of assisted reproductive treatment outcome, which is of key importance to patients and their

TABLE 1

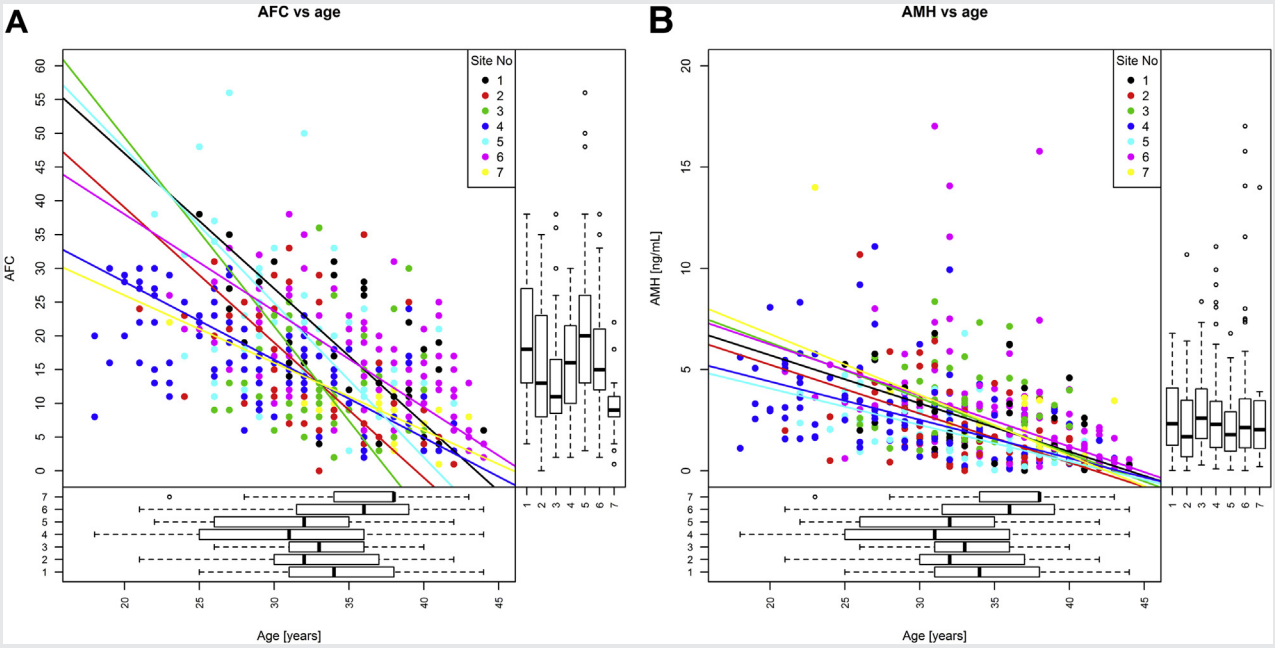
Demographic characteristics of subjects included in the analysis, overall and by study site.

		Site number						
Characteristics	**Overall (N = 451)**	**1 (n = 37)**	**2 (n = 55)**	**3 (n = 59)**	**4 (n = 111)**	**5 (n = 57)**	**6 (n = 115)**	**7 (n = 17)**
Age (y)								
Mean (95% CI)	32.84 (32.31–33.38)	34.16 (32.54–35.79)	32.76 (31.43–34.10)	33.12 (32.15–34.08)	30.17 (28.89–31.45)	31.16 (29.79–32.53)	35.23 (34.25–36.21)	36.24 (33.67–38.80)
SD	5.78	4.87	4.94	3.7	6.82	5.16	5.31	4.99
Min–Max	18.00–44.00	25.00–44.00	21.00–42.00	26.00–40.00	18.00–44.00	22.00–42.00	21.00–44.00	23.00–43.00
Age group (y)								
18–29	123 (27.27)	7 (18.92)	13 (23.64)	9 (15.25)	51 (45.95)	25 (43.86)	16 (13.91)	2 (11.76)
30–34	148 (32.82)	13 (35.14)	22 (40.00)	32 (54.24)	28 (25.23)	16 (28.07)	34 (29.57)	3 (17.65)
35–39	120 (26.61)	11 (29.73)	13 (23.64)	15 (25.42)	22 (19.82)	13 (22.81)	38 (33.04)	8 (47.06)
40–45	60 (13.30)	6 (16.22)	7 (12.73)	3 (5.08)	10 (9.01)	3 (5.26)	27 (23.48)	4 (23.53)
Race								
White	414 (91.80)	32 (86.49)	46 (83.64)	55 (93.22)	109 (98.20)	47 (82.46)	110 (95.65)	15 (88.24)
Black	15 (3.33)	0 (0.00)	5 (9.09)	1 (1.69)	0 (0.00)	9 (15.79)	0 (0.00)	0 (0.00)
Asian	16 (3.55)	5 (13.51)	0 (0.00)	3 (5.08)	0 (0.00)	1 (1.75)	5 (4.35)	2 (11.76)
Other	6 (1.33)	0 (0.00)	4 (7.27)	0 (0.00)	2 (1.80)	0 (0.00)	0 (0.00)	0 (0.00)
Smoking habit								
Current	99 (21.95)	3 (8.11)	8 (14.55)	8 (13.56)	52 (46.85)	16 (28.07)	11 (9.57)	1 (5.88)
Past	80 (17.74)	6 (16.22)	13 (23.64)	2 (3.39)	8 (7.21)	11 (19.30)	34 (29.57)	6 (35.29)
Never	272 (60.31)	28 (75.68)	34 (61.82)	49 (83.05)	51 (45.95)	30 (52.63)	70 (60.87)	10 (58.82)
Female infertility								
No	309 (68.51)	20 (54.05)	28 (50.91)	27 (45.76)	90 (81.08)	30 (52.63)	109 (94.78)	5 (29.41)
Yes	142 (31.49)	17 (45.95)	27 (49.09)	32 (54.24)	21 (18.92)	27 (47.37)	6 (5.22)	12 (70.59)
BMI								
Mean (95% CI)	24.30 (23.86–24.74)	25.72 (23.74–27.69)	24.03 (22.61–25.45)	22.88 (21.97–23.79)	23.07 (22.46–23.69)	25.32 (23.78–26.87)	25.46 (24.53–26.38)	23.84 (21.88–25.81)
SD	4.77	5.91	5.27	3.49	3.28	5.82	5.02	3.82
Min–Max	16.70–52.70	16.70–40.40	17.70–52.70	18.20–34.10	17.80–33.50	17.70–41.00	17.80–47.30	18.20–33.50

Note: Percentage of total in parentheses. CI = confidence interval; SD = standard deviation.

Anderson. Automated AMH assay in ovarian assessment. Fertil Steril 2015.

FIGURE 1



The relationships between (**A**) AFC and (**B**) AMH with age, with data from each site shown separately in different colors. To get a more structured view of this data among the different centers, the robust Passing-Bablok regression was chosen to exemplify the mean relationship within each center in this plot. Site-specific AFC/AMH and age distributions are shown in the box and whiskers plots, where the lower and upper limits of the boxes show the lower and upper quartile, the line within the box the median, and the whiskers up to 1.5 times the interquartile range.

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clinical teams. Although live birth is the ultimate positive outcome, prediction of ovarian response is also of great value for identifying women who are likely to respond poorly, thus allowing appropriate counseling in advance, and for identifying women likely to show an excessive response, thus predicting a potential for OHSS. Thus, in both groups management strategies can be tailored to optimize outcome and minimize risk (4).

Both AFC and AMH have specific characteristics and thus advantages and disadvantages as biomarkers. Because AFC is widely available, it has the advantage of immediacy, but standardization is a difficulty. Ultrasound equipment varies among centers, and there have been progressive increases in resolution and thus image quality over recent years. This has led to substantial changes in what is regarded as a "normal" AFC, as well as in what might be used as a diagnostic criterion in, for example, polycystic ovarian syndrome (5). There is also well-recognized, substantial interobserver variation (17), compounding the variations among women and across the menstrual cycle (18); as a result, assessment in the early follicular phase is required, as performed in this study. Although AMH may show less intraindividual and interindividual variation than AFC (19–21), the lack of standardization of the calibrators among the different manufacturers is also a significant issue, as are other methodologic problems that have affected reproducibility (12). Previous assays have also all been manual plate-based formats, with inherent susceptibility to variation within and between laboratories and in lot-to-lot variation. The technical characteristics of the fully automated AMH assay used in this study have been recently described, indicating that it may address many of the issues with the measurement of this hormone (13, 22).

The present hormone analyses were all performed in a single academic laboratory, with a very similar technical performance for AMH to that previously described. There was no significant variation in AMH distribution between centers, although there was significant variation in AFC. Likewise, although both AMH and AFC showed the expected inverse relationship with age, there was significant variation in that relationship among centers for AFC but not for AMH. These analyses may indicate that AMH measured by this new assay is a more reliable indicator of the ovarian reserve than AFC. The full automation of the AMH assay removes operator-related variation; the centralization of hormone analysis

TABLE 2

Agreement table for antral follicle count (AFC) groups and new defined antimüllerian hormone (AMH) groups.

AMH group	AFC 0–7	AFC 8–15	AFC > 15	N
AMH ≤ 0.681	43 (63.2%)	22 (32.4%)	3 (4.4%)	68
0.681 < AMH ≤ 2.27	20 (12.0%)	95 (56.9%)	52 (31.1%)	167
AMH > 2.27	3 (1.4%)	52 (24.1%)	161 (74.5%)	216
N	66	169	216	451

Note: Percentages refer to AMH group numbers (AMH values in ng/mL).

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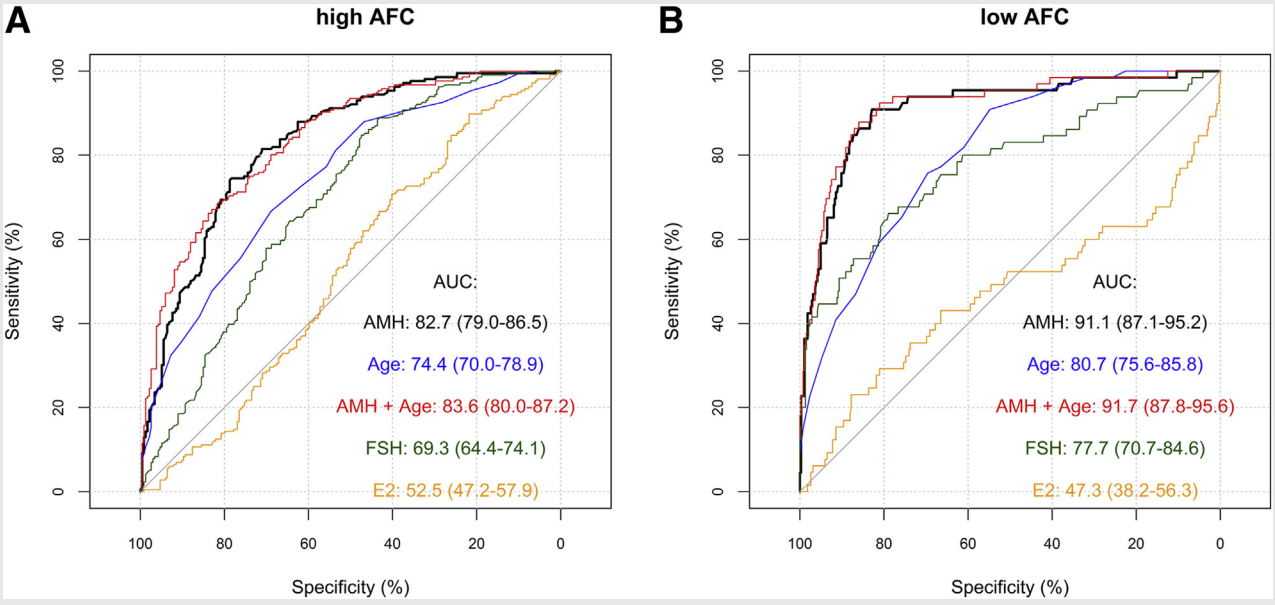
FIGURE 2



ROC curves for classification of (**A**) low AFC and (**B**) high AFC, by AMH, FSH, E_2 and age. For low AFC, n = 66 patients with AFC ≤7 versus 385 subjects AFC >7. For high AFC, n = 216 subjects with AFC >15 versus 235 patients AFC ≤15.

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removes a further source of variability, and thus this may not exactly reflect the situation where individual sites perform hormone analysis. The possibility of centralization is an inherent advantage of hormone analysis over ultrasound-based analysis: recording images and their offline and centralized analysis are possible in principle (17), but they are very time consuming, so one of the key advantages of AFC, its immediacy, is lost.

Although AMH and AFC are sometimes regarded as interchangeable, there are emerging data suggesting that AMH is a more reproducible measure of the ovarian response to stimulation. In an analysis of potential markers to predict ovarian response in a randomized controlled trial, AFC was not found to be predictive (23). In contrast, AMH was predictive both of number of retrieved oocytes and of poor versus excessive response. Similarly, in another randomized controlled trial, AMH but not AFC predicted oocyte yield after ovarian stimulation (24). These findings have led to discussion of the limitations of AFC for the prediction of ovarian response, particularly in multicenter trials (25). Although the present data do not include assessment of ovarian response, the finding of significant intercenter variability in the relationship between AFC and age but not between AMH and age is consistent with this growing recognition of difficulties in standardizing AFC assessment among centers.

Further characterization of the relationship between AFC and AMH showed good performance in an agreement table and in ROC analyses. These results are similar to those previously reported using other AMH assays (4, 6) and support the value of AMH across the dynamic range found in women of ages across the reproductive life span.

In conclusion, these data demonstrate the clinical performance of the new fully automated Elecsys AMH assay in the analysis of the ovarian reserve in women of reproductive age. The expected relationships with age and AFC were observed, with evidence of much lower variability in the determination of AMH compared with AFC. This supports the use of the automated AMH assay in a range of contexts in reproductive medicine such as in physiologic, therapeutic, and potentially pathologic investigations.

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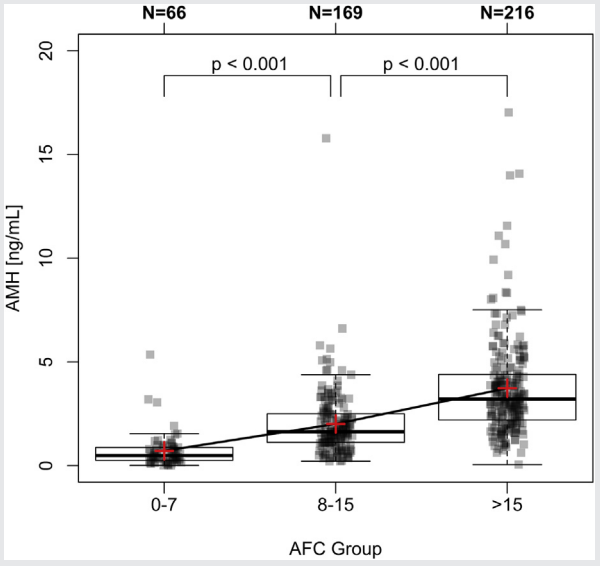
SUPPLEMENTAL FIGURE 1



Distribution of AMH (ng/mL) in different AFC groups for all sites. The *P* values in the graph refer to *t* tests of AMH means between the AFC 0–7 and 8–15 group and between the 8–15 and >15 group.

Anderson. Automated AMH assay in ovarian assessment. Fertil Steril 2015.

SUPPLEMENTAL TABLE 1

Characteristics of ultrasonography equipment used for transvaginal sonography at study sites.

	Study site						
Equipment	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Manufacturer	GE	Siemens	Toshiba	GE	GE	Toshiba	GE
Device type	LOGIQ P3	Acuson X300	Nemio XG	Voluson 730 ProV	Voluson E8	Nemio XG Mk2	Voluson S8
Year of manufacture	2009	2008	2011	2005	2010	2008	2012
Probe frequency	8 MHz	4–9 MHz	7.5 MHz	3.7–9.3 MHz	4–8 MHz	6 MHz	8 MHz
Probe resolution	2 mm	2 mm	< 2 mm	2 mm	1 mm	1 mm	2 mm
Yearly maintenance	Yes	Yes	Yes	Yes	Yes	Yes	Yes

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SUPPLEMENTAL TABLE 2

Antral follicle count (AFC), antimüllerian hormone (AMH), follicle-stimulating hormone (FSH), and estradiol (E_2) data in study participants, overall and by study site.

		Site number						
	Overall (N = 451)	**1 (n = 37)**	**2 (n = 55)**	**3 (n = 59)**	**4 (n = 111)**	**5 (n = 57)**	**6 (n = 115)**	**7 (n = 17)**
AFC group								
0–7	66 (14.63)	5 (13.51)	13 (23.64)	10 (16.95)	20 (18.02)	2 (3.51)	12 (10.43)	4 (23.53)
8–15	169 (37.47)	7 (18.92)	18 (32.73)	32 (54.24)	34 (30.63)	18 (31.58)	49 (42.61)	11 (64.71)
>15	216 (47.89)	25 (67.57)	24 (43.64)	17 (28.81)	57 (51.35)	37 (64.91)	54 (46.96)	2 (11.76)
AFC [n]								
Mean (95% CI)	16.17 (15.37–16.96)	19.03 (16.06–22.00)	15.31 (12.85–17.77)	13.39 (11.42–15.36)	15.63 (14.20–17.06)	21.30 (18.39–24.21)	16.02 (14.69–17.35)	9.71 (7.12–12.29)
SD	8.58	8.91	9.1	7.55	7.62	10.96	7.2	5.02
Min–Max	0.00–56.00	4.00–38.00	0.00–35.00	2.00–38.00	2.00–30.00	3.00–56.00	2.00–38.00	1.00–22.00
AMH [ng/mL]								
Mean (95% CI)	2.64 (2.44–2.85)	2.68 (2.06–3.30)	2.27 (1.72–2.83)	3.01 (2.53–3.49)	2.65 (2.25–3.05)	2.12 (1.72–2.52)	2.86 (2.34–3.39)	2.74 (1.31–4.35)
SD	2.26	1.86	2.06	1.83	2.14	1.51	2.84	3.13
Min–Max	0.01–17.02	0.02–6.79	0.01–10.68	0.29–8.36	0.10–11.08	0.05–6.79	0.01–17.02	0.21–13.99
FSH [IU/L]								
Mean (95% CI)	7.44 (7.10–7.79)	8.21 (5.85–10.57)	8.01 (6.97–9.06)	6.98 (6.49–7.46)	7.43 (6.84–8.03)	7.56 (6.39–8.73)	7.18 (6.65–7.71)	7.01 (4.81–9.22)
SD	3.74	7.08	3.83	1.85	3.17	4.42	2.85	4.3
Min–Max	0.51–45.94	3.75–45.94	3.81–26.00	3.67–14.35	1.37–22.21	4.34–36.38	0.51–18.61	0.68–22.32
E_2 [pmol/L]								
Mean (95% CI)	158 (150–165)	145 (120–169)	166 (145–187)	156 (137–175)	153 (135–171)	158 (138–178)	156 (142–170)	204 (127–282)
SD	84.34	72.86	77.7	71.76	94.76	75.18	76.61	150.25
Min–Max	18.35–684	18.35–354	56.59–396	62.32–348	18.35–684	52.44–461	30.17–631	26.31–655

Note: Percentage of total in parentheses. CI = confidence interval; SD = standard deviation.

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SUPPLEMENTAL TABLE 3

Correlation between AFC and AMH over all sites and for individual sites.

Correlation	All	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
Spearman	0.68	0.71	0.86	0.49	0.87	0.74	0.62	0.75
P value (Spearman)	< .001	< .001	< .001	< .001	< .001	< .001	< .001	< .001
Intercept	−0.25	−0.51	−0.27	0.22	−0.64	−0.29	−0.76	−1.32
Slope	0.16	0.14	0.16	0.20	0.20	0.11	0.21	0.36
N	451	37	55	59	111	57	115	17

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